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QUANTITATIVE ASSAY OF PSITTACOSIS VIRUS BY THE FLUORESCENT CELL-COUNTING TECHNIQUE

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QUANTITATIVE ASSAY OF PSITTACOSIS VIRUS BY THE FLUORESCENT CELL-COUNTING TECHNIQUE

Nicholas Hahon

Robert M. Nakamura

Aerobiology Division
DIRECTOR OF BIOLOGICAL RESEARCH

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ABSTRACT

The studies described here indicate that the fluorescent cell-counting technique provides a rapid, precise, reproducible, and sensitive procedure for the assay of infective particles of psittacosis virus. The efficiency of virus adsorption onto coverslip cultures of McCoy cells was markedly increased by the use of centrifugal force; assay values were 1.0 log higher than those obtained after stationary incubation. With the former procedure, the rate of cell-virus contact was independent of the volume of inoculum. Incubation for 20 to 22 hours was optimal for enumerating fluorescent viral particles in stained cell cultures. Virus assay values were not affected unduly by the incubation of infected cell cultures at temperatures ranging from 32° to 39°C. The number of fluorescent cell-infecting units was a linear function of virus concentration; the distribution of infected cells on coverslip cultures was random. The reduction of cell-infecting units of psittacosis virus by dilutions of specific antiserum suggests the possible usefulness of the procedure for detecting and measuring levels of serum-neutralizing antibody.

The demonstration that the concentration of psittacosis virus in aerosols can be determined within 24 hours exemplifies the rapidity and applicability of the fluorescent cell-counting technique.

I. INTRODUCTION

The fluorescent cell-counting technique, introduced by Wheelock and Tamm[1] for the assay of Newcastle disease virus, has been applied successfully for the titration of adenovirus[2] and human cytomegalovirus.[3] The technique provides a highly reproducible and rapid assay for infective virus particles by the enumeration of cells containing virus antigen that is produced, depending on the strain of virus inoculum employed, within 10 to 48 hours. In sensitivity, the method compared favorably with the different animal and tissue culture systems that were employed to estimate the concentration of the aforesaid viruses. In view of these marked advantages, the feasibility of using this procedure for the assay of psittacosis virus was investigated.

This report describes the application and standardization of the fluorescent cell-counting technique for the quantitative assay of psittacosis virus.

II. MATERIALS AND METHODS

A. VIRUS

The Borg strain of psittacosis virus was used in these studies; its history has been recorded elsewhere.[4] Stock suspensions of virus were prepared by infecting monolayers of McCoy cells with virus that had been passed four times previously in this cell line. After incubation for 72 hours at 35°C, tissue culture fluids were harvested, clarified by low-speed centrifugation, dispensed into glass vials, and stored at -60°C. Stock suspensions of virus assayed by the method of mouse intracerebral inoculation contained approximately $10^{7.0}$ LD_{50} per milliliter.

B. CELL LINE

The established cell line, McCoy, derived from human synovial tissue[5] was used for virus assay. A culture was obtained from Dr. Francis B. Gordon, Naval Medical Research Institute, Bethesda, Md. Preliminary experiments employing high multiplicities of virus to cells indicated that approximately 99 per cent of the cells were susceptible to infection by psittacosis virus.

C. CELL CULTIVATION

Nutrient medium for the cell line consisted of mixture 199 containing 0.5 per cent lactalbumin hydrolyzate, 10 per cent heat-inactivated calf serum, 50 micrograms of streptomycin, and 75 micrograms of kanamycin. Cells were maintained in mixture 199 and 5 per cent calf serum. For virus assay, cells were cultivated on circular coverslips (15-millimeter diameter) inserted in flatbottomed glass vials (18 by 100 millimeters). One milliliter of cell suspension, containing 1×10^5 to 3×10^5 cells, was introduced onto coverslips that were then incubated for 24 hours at 35°C or until a complete cell monolayer was formed. Coverslip cultures were washed twice with two milliliters of maintenance medium prior to the addition of virus inoculum.

D. VIRUS ASSAY

Virus dilutions were prepared in maintenance medium and introduced in 0.2-milliliter volumes directly into vials containing coverslip cell cultures. Routinely, virus adsorption was carried out by centrifugation at 500g for 30 minutes at 21° to 23°C. For this procedure, vials were placed in slotted cups containing tube adapters, sealed with a screw-dome cover, and mounted on a four-place, pin-type head. Centrifugation was performed in an International centrifuge, size 2, model V. Coverslip cultures were rinsed twice with maintenance medium following adsorption of virus; one milliliter of the medium was added then to each vial. After incubation for 20 to 22 hours at 35°C, coverslips were rinsed twice with cold PBS, fixed with cold (-60°C) acetone, and either prepared immediately for immunofluorescent staining and cell-counting or stored at -60°C for subsequent examination. Fixed cell cultures stored at -60°C for as long as two weeks showed no decrease in fluorescence on staining.

E. ANTISERUM CONJUGATE AND TECHNIQUE OF IMMUNOFLUORESCENT STAINING

Psittacosis virus antiserum obtained from immunized turkeys was conjugated with fluorescein isothiocyanate by the method of Riggs *et al.*[8] The conjugate was adsorbed twice with liver, kidney, heart, and muscle powder of rabbit origin and once with spleen and bone marrow powder. For staining, the conjugate was used as a 1:10 dilution.

The direct fluorescent antibody technique was employed to obtain immunofluorescence of infected cells. Infected cell monolayers, fixed with cold (-60°C) acetone previously, were washed three times with PBS and stained with the conjugate for 30 minutes. Coverslip cultures were rinsed then in three changes of PBS to remove excess conjugate and mounted in ten per cent glycerol in PBS.

F. FLUORESCENCE MICROSCOPY

Coverslip cultures were examined with a Zeiss fluorescence microscope equipped with a 200-watt Osram lamp, Schott UG-2 and UG-5 transmitting filters, and a Schott GG-4 barrier filter.

G. FLUORESCENT CELL-COUNTING AND CALCULATIONS

The number of microscopic fields contained in the area of a 15-millimeter coverslip was 2208 with the optical system employed. Each field contained 150 ± 25 cells. For each coverslip culture, 100 microscopic fields were examined for fluorescent cells. To calculate the number of cell-infecting units (CIU) of virus per milliliter, the average number of fluorescent cells per field was multiplied by the number of fields per coverslip, the reciprocal of the dilution of virus inoculum, and a volume factor (for conversion to milliliters).

III. RESULTS

A. CONDITIONS FOR VIRUS ADSORPTION

1. Centrifugal Force versus Stationary Incubation

The efficiency of various procedures for the adsorption of psittacosis virus particles onto cell monolayers was investigated. Virus adsorption was carried out at different conditions of centrifugation and stationary incubation. Two-tenths milliliter of a 10^{-3} dilution of virus stock suspension was introduced into several vials containing coverslip cell monolayers that were subjected, in groups of three, to different experimental treatments. Results in Table I reveal that the number of cell-infecting units of virus were markedly higher after the application of centrifugal force than after stationary incubation during the period of virus adsorption.

2. Rates

The rate of virus adsorption onto cell monolayers was determined during stationary incubation (35°C) and centrifugation (500g). The latter condition was selected arbitrarily because the efficiency of virus adsorption appeared comparable at all centrifugal speeds that were tested initially. For these experiments, 0.5 milliliter of a 10^{-3} dilution of virus was added to vials containing coverslip cultures. During the period of virus adsorption, vials were removed at designated intervals and 0.25 milliliter of residual inoculum from each vial was introduced onto additional cell monolayers to measure the quantity of unadsorbed virus.

TABLE I. EFFECT ON THE ASSAY OF PSITTACOSIS VIRUS OF VARIED CONDITIONS FOR ADSORPTION OF VIRUS

Condition for Virus Adsorption		10^7 CIU per Milliliter[a]
Centrifugation	250g, 1 hour, 23°C	2.2
	500g, 1 hour, 23°C	2.2
	800g, 1 hour, 23°C	1.9
	1300g, 1 hour, 26°C	1.7
Stationary incubation	1 hour, 23°C	0.06
	2 hours, 23°C	0.13
	2 hours, 35°C	0.12

a. Cell-infecting units. Mean of three determinations.

Residual inocula derived from cell monolayers that had been centrifuged or held at stationary incubation previously were adsorbed at 500g for one hour at 23°C and two hours at 35°C, respectively. Following the designated periods for virus adsorption for both initial and residual inocula, all coverslip cultures were rinsed twice with maintenance medium and incubated with fresh medium for 20 hours at 35°C.

The per cent of virus adsorbed during each interval with each procedure is depicted in Figure 1. Within 15 to 30 minutes, more than 98 per cent of the virus was adsorbed during centrifugation, whereas approximately 55 per cent was adsorbed during stationary incubation for two hours. Since the efficiency and rapidity of virus adsorption onto cell monolayers attained by the use of centrifugal force was clearly superior to results with stationary incubation, centrifugation at 500g for 30 minutes at room temperature was employed as the procedure for virus adsorption in all subsequent experiments.

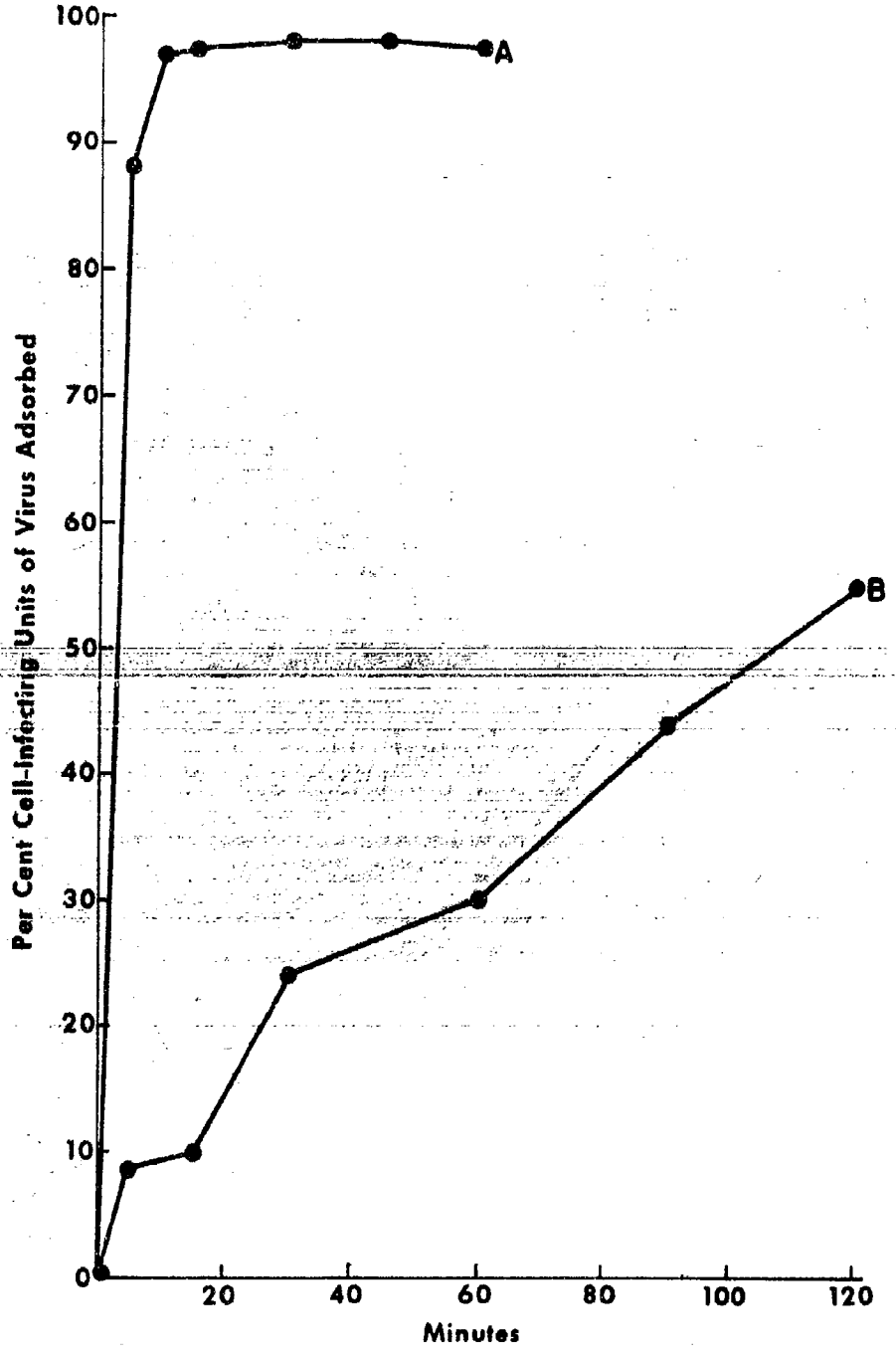


Figure 1. Adsorption of Psittacosis Virus onto Coverslip Cultures of McCoy Cells by (A) Centrifugal Force (500g, 23°C) and at (B) Stationary Incubation (35°C).

3. Volume of Inoculum

An experiment was performed to ascertain the efficiency of centrifugation for infecting cell monolayers from different volumes of inoculum. Adsorption from different volumes of a 10^{-3} dilution of virus inoculum onto cell monolayers was carried out in the standard manner and then the monolayers were incubated for 22 hours at 35°C. Results in Table II reveal a proportional increase in the number of cell-infecting units of virus with volume of inoculum from 0.1 to 1.0 milliliter. Although the value obtained with a 2.0-milliliter volume was not strictly proportional, in all probability an extension of the centrifugation time or the use of greater centrifugal force would increase the efficiency of virus adsorption from this volume of inoculum. Proportionality between different volumes of inoculum and infected cell counts was not demonstrable when virus adsorption was carried out by stationary incubation. It appears, within practical limits, that the rate of cell-virus contact is independent of the volume of inoculum when adsorption is carried out with the aid of centrifugal force.

TABLE II. PROPORTIONALITY BETWEEN VOLUME OF INOCULUM AND CELL-INFECTING UNITS OF PSITTACOSIS VIRUS

Volume, ml	CIU[a] per 100 Fields
0.1	33
0.2	73
0.5	163
1.0	358
2.0	569

a. Cell-infecting units. Mean of two determinations.

B. INCUBATION PERIOD

1. Length

The incubation period, defined in this assay system as the interval between virus inoculation and the development of recognizable quantities of viral antigen in cell cultures, is an integral part of the fluorescent cell-counting procedure. Ideally, this period should be terminated before newly synthesized virus particles are released (to prevent secondary infection of cells) but should be sufficient to insure the primary formation of substantial amounts of viral antigen within infected cells. To establish an appropriate period of incubation, cell monolayers were infected with an equivalent concentration of virus inocula and adsorbed in the prescribed manner. At designated intervals of incubation at 35°C, three infected cell cultures were stained with fluorescent antibody and examined.

Minute, intracytoplasmic particles exhibiting faint fluorescence, suggestive of viral antigen, were observed as early as four to seven hours after infection. At 12 hours, the fluorescent particles had increased in size, but they were difficult to detect consistently (Figure 2). From 18 to 22 hours after infection, fluorescent inclusions were plainly visible and infected cells could be counted easily (Figure 3). Inclusions were larger and often segmented at 24 hours after infection (Figure 4) and, occasionally, a few appeared to have burst. Cell monolayers incubated for 18, 20, 22, and 24 hours after infection had an equivalent number of cells that exhibited fluorescence. Thirty hours after infection, inclusions were very large and many showed signs of rupture. Usually, minute, granular, fluorescent particles were seen in the vicinity of these inclusions (Figure 5). The fluorescence of inclusions that had burst was markedly reduced. Based on these sequential observations on the development of viral antigen within cells, the optimal period for incubation of infected cell monolayers was established as 20 to 22 hours.

Cell cultures treated with virus inactivated by ultraviolet irradiation or heat (56°C, one hour) exhibited no fluorescence on staining.

2. Temperature

The influence of different temperatures of incubation on the assay of virus is shown in Table III. Uniform values for virus assays were obtained when incubation periods (22 hours) were carried out at temperatures of 35°, 37°, and 39°C. Inclusions were large, distinct, and could be counted easily when infected cell cultures were incubated at these temperatures. The slow formation of viral antigen at 32°C, indicated by the appearance of inclusions that were small and sometimes difficult to enumerate, may account for the slightly lower value obtained at this temperature.

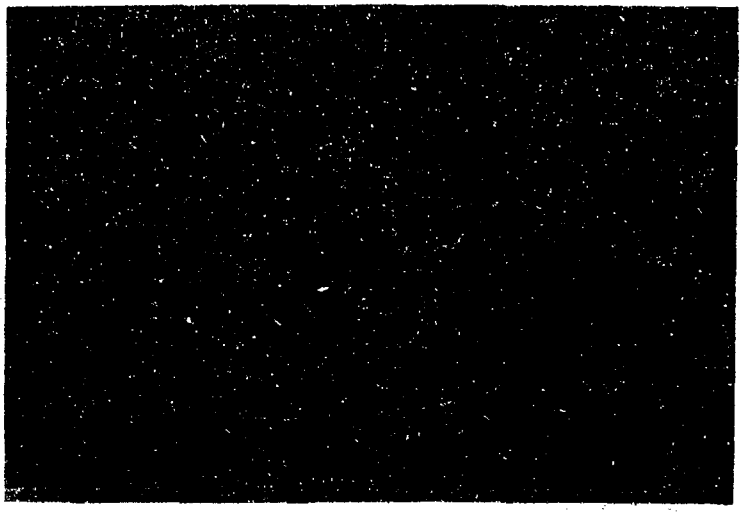


Figure 2. Fluorescent Psittacosis Virus Antigen in Coverslip Cultures of McCoy Cells. Minute particles seen 12 hours after infection. (320X)

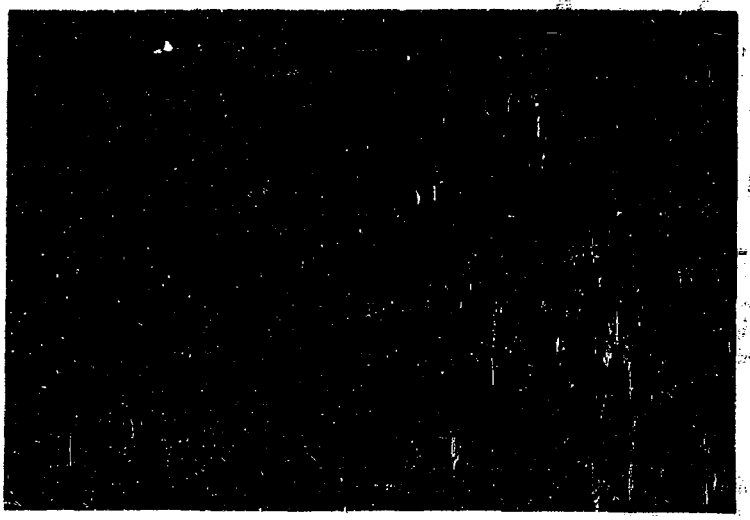


Figure 3. Fluorescent Psittacosis Virus Antigen in Coverslip Cultures of McCoy Cells. Large fluorescent particles 22 hours after infection. (320X)

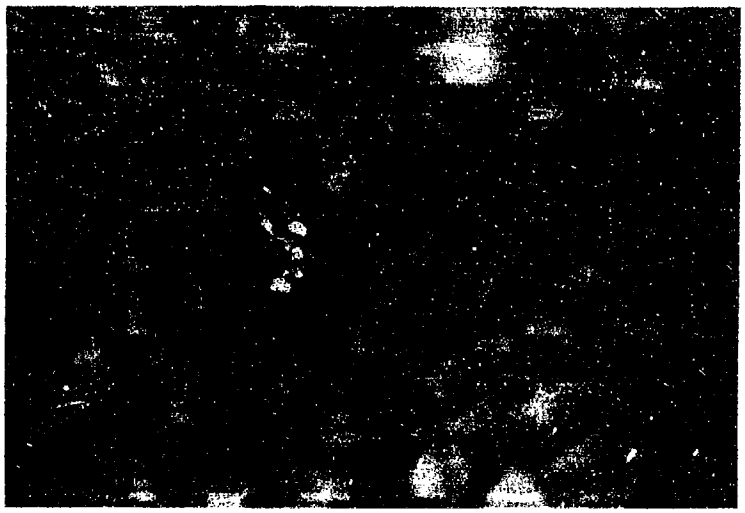


Figure 4. Fluorescent Psittacosis Virus Antigen in Coverslip Cultures of McCoy Cells. Segmented viral inclusion 24 hours after infection. (320X)

Figure 5. Fluorescent Psittacosis Virus Antigen in Coverslip Cultures of McCoy Cells. Ruptured viral inclusion 30 hours after infection, exhibiting diminished fluorescence. Note minute particles in vicinity of inclusion. (320X)

TABLE III. EFFECT ON THE ASSAY OF PSITTACOSIS VIRUS OF DIFFERENT TEMPERATURES OF INCUBATION FOR INFECTED MCCOY CELL MONOLAYERS

Temperature, °C	10^7 CIU[a] per ml
32	1.1
35	1.6
37	1.6
39	1.4

a. Cell-infecting units. Mean of three determinations.

C. LINEARITY BETWEEN VIRUS CONCENTRATION AND CELL-INFECTING UNITS

Results in Figure 6 depict a linear relationship between twofold dilutions of virus over a range of 1.8 log units and the number of cell-infecting units of virus. These data suggest that each fluorescent cell was the consequence of infection by a single infective virus particle.

D. RANDOM DISTRIBUTION OF INFECTED CELLS

Two hundred random fields were examined for fluorescent cells on each of four coverslip cultures previously infected with virus. The frequency of fields containing fluorescent cells was compared with the theoretical Poisson distribution. A representative determination shown in Figure 7 indicates that the observed frequencies of infected cells corresponded closely to the theoretical frequencies. There was no evidence of a significant departure from Poisson variation at the 0.05 level in any of the four sets of data using the X^2 test of goodness of fit. Since the mean and the variance of the Poisson distribution are the same, the ratio of the sample variance to the sample mean is a measure of the extent of departure from Poisson variation. The mean ratio of the four sets of data was approximately 1.19. These data are indicative of a random distribution of infected cells.

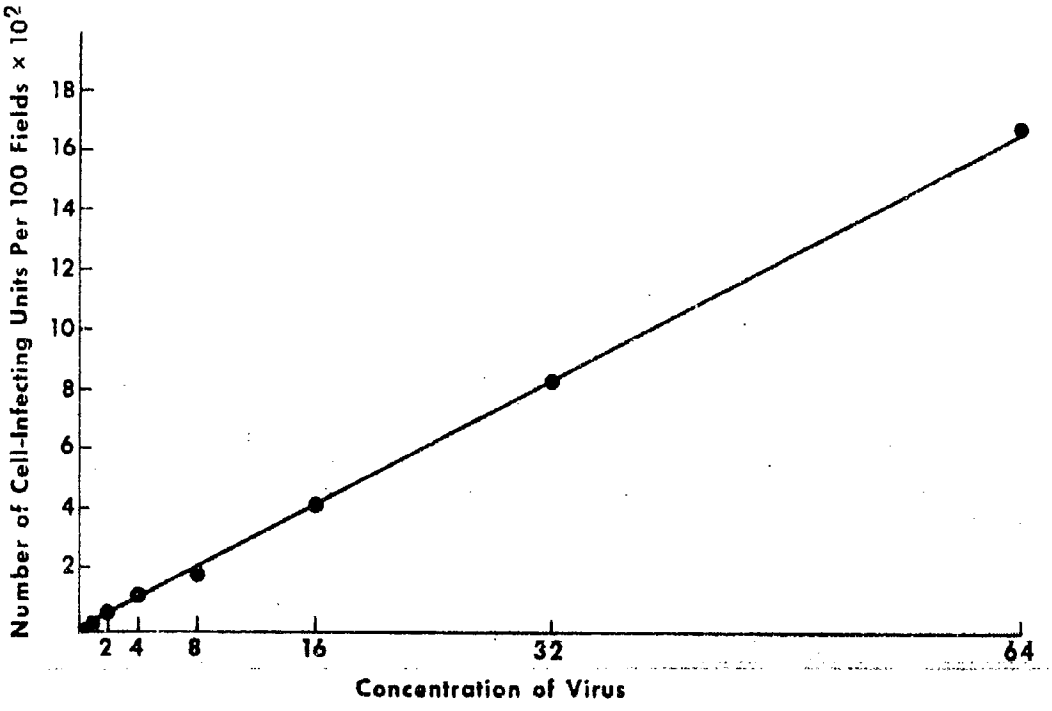


Figure 6. Linear Function Between the Number of Cell-Infecting Units and Concentration of Psittacosis Virus. Points are the mean of two determinations.

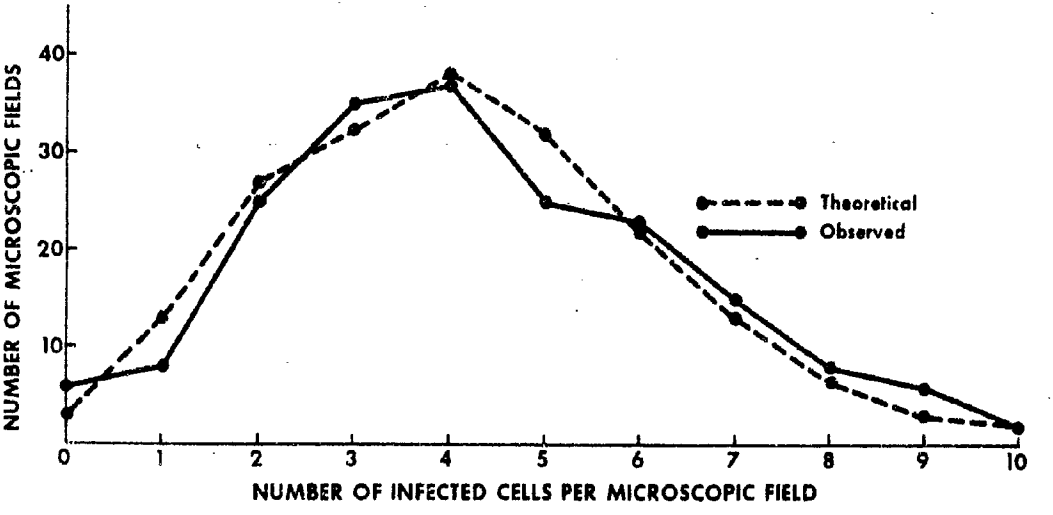


Figure 7. Frequency Distribution of McCoy Cells Infected with Psittacosis Virus.

E. PRECISION, SENSITIVITY, AND REPRODUCIBILITY OF THE ASSAY

Twelve determinations were made to estimate the precision of the fluorescent cell-counting technique. Cell cultures were infected and incubated in the prescribed manner. The number of cell-infecting units of virus per milliliter of inoculum ranged from 1.4×10^7 to 2.3×10^7 with a mean of 1.7×10^7. The standard deviation was 0.29.

The sensitivity of the cell-counting procedure was compared with that of three other methods that employ dilution endpoints for estimating the concentration of psittacosis virus. Cell-infecting units of virus were converted to logarithms to facilitate a comparison of the data shown in Table IV. The fluorescent cell-counting procedure, within the limits of the doses employed, was equivalent in sensitivity to two of the assay methods, intracerebral inoculation of mice and yolk-sac inoculation of chick embryonated eggs, and appeared superior to the cell-culture system using cytopathic changes as the index of virus infectivity.

The reproducibility of the fluorescent cell-counting technique employing several dilutions of virus inoculum in each of four determinations is shown in Table V. The data attest to the reproducibility of the assay at the concentration levels employed.

F. NEUTRALIZATION OF CELL-INFECTING UNITS OF VIRUS

A serum neutralization test was carried out using dilutions of psittacosis antiserum, each of which was mixed with a standard concentration of virus inoculum. Results in Table VI show that various dilutions of antiserum produced a marked reduction in the number of cell-infecting units of virus.

G. ASSAY OF VIRUS AEROSOLS

The usual procedure for determining the viability and concentration of viral agents in aerosols is to introduce portions or dilutions of the impinger fluids into susceptible hosts and to note the manifestation of responses indicative of virus infection. The intracerebral inoculation of mice exemplifies this method of assessment. It was compared with the fluorescent cell-counting technique for determining the concentration of psittacosis virus in aerosols. Details and results of several tests that were made to compare the two techniques are shown in Table VII. These data indicate that the fluorescent cell-counting technique was comparable in sensitivity, if not superior, to the former procedure for estimating the concentration of psittacosis virus. The time required to assess the aerosols was another important consideration in the comparison of techniques. Virus aerosols were assessed within 24 hours by the fluorescent cell-counting technique, whereas 14 days were required by intracerebral inoculation of mice.

TABLE IV. COMPARISON OF DIFFERENT METHODS FOR THE ASSAY OF PSITTACOSIS VIRUS

Assay Number	CIU[a] per ml	$MICLD_{50}$[b] per ml	ELD_{50}[c] per ml	$TCID_{50}$[d] per ml
1	7.1	7.3	7.2	6.5
2	7.2	6.9	7.0	7.0
3	7.2	7.3	7.0	7.0
4	7.3	7.0	7.2	7.1
5	7.3	7.3	7.2	7.0
6	7.3	7.4	7.1	7.0
7	7.2	7.1	6.8	6.5
8	7.1	7.2	6.8	7.0
9	7.2	7.0	6.7	6.5
10	7.2	7.0	7.2	6.5
11	7.2	7.2	7.4	6.5
Mean	7.2	7.1	7.0	6.7
Standard Deviation	±0.07	±0.17	±0.22	±0.27
Standard error of mean	0.02	0.04	0.07	0.08

a. Cell-infecting units of virus (log_{10}) determined in 24 hours.
b. Reciprocal of LD_{50} (log_{10}) mouse intracerebral inoculation, 14-day observation period.
c. Reciprocal of LD_{50} (log_{10}) yolk-sac inoculation of seven-day chick embryos, 10-day observation period.
d. Reciprocal of ID_{50} (log_{10}) cytopathic changes in stationary tube cultures of McCoy cells, seven-day observation period.

TABLE V. REPRODUCIBILITY OF DETERMINATIONS OF CELL-INFECTING UNITS (CIU) OF PSITTACOSIS VIRUS

Assay Number	10^2 Fluorescent Cells/100 Fields, Dilution of Virus in Inoculum					10^7 CIU per ml
	2.5×10^{-2}	1.25×10^{-2}	8.3×10^{-3}	6.2×10^{-3}	2.5×10^{-3}	
1	5.7	2.3	1.5	1.2	0.4	1.3
2	4.5	2.1	1.1	1.1	0.4	1.1
3	4.2	2.2	1.0	1.1	-	1.1
4	5.0	2.4	1.2	1.1	0.4	1.2
Mean	4.8	2.2	1.2	1.1	0.4	1.2

TABLE VI. NEUTRALIZATION OF CELL-INFECTING UNITS OF PSITTACOSIS VIRUS BY DILUTIONS OF SPECIFIC ANTISERUM

Final dilution of antiserum[a]	CIU per 100 fields[b]
1:2	0
1:10	6
1:100	96
1:500	184
1:1000	197
Control	227

a. Dilutions of antiserum were mixed with equal volumes of 4.0×10^{-2} dilution of virus suspension, incubated for 2½ hours at 25°C, and inoculated in 0.2-milliliter volumes onto cell monolayers. Virus adsorption was carried out by centrifugation, followed by removal of excess inoculum and subsequent incubation of cell cultures for 22 hours at 35°C.

b. Cell-infecting units of virus. Mean of two determinations.

TABLE VII. A COMPARISON OF ASSAY TECHNIQUES FOR ESTIMATING THE CONCENTRATION OF PSITTACOSIS VIRUS IN AEROSOLS

Test Number	Impinger Assay: Fluorescent Cell-Counting					Impinger Assay: Intracerebral Inoc. of Mice				
	CONCENTRATION PER IMPINGER FLUID[a] / PER CENT RECOVERY									
	Cloud Age, minutes				Per Cent Decay Per Minute (0-90 min.)	Cloud Age, minutes				Per Cent Decay Per Minute (0-90 min.)
	0	30	60	90		0	30	60	90	
1	1.3×10^5 [b] / 1.7	3.3×10^4 / 0.43	1.9×10^4 / 0.25	8.8×10^3 / 0.11	3.0	5.0×10^4 [c] / 3.0	3.2×10^3 / 0.16	2.0×10^3 / 0.10	2.0×10^3 / 0.10	3.4
2	1.5×10^5 / 1.9	6.1×10^4 / 0.79	1.9×10^4 / 0.25	5.5×10^3 / 0.07	3.6	1.6×10^4 / 0.84	- [d]	2.0×10^3 / 0.10	-	-
3	1.3×10^5 / 1.7	3.7×10^4 / 0.48	1.1×10^4 / 0.14	8.8×10^3 / 0.11	3.0	1.2×10^4 / 1.6	4.0×10^3 / 0.20	8.0×10^2 / 0.04	2.0×10^3 / 0.10	3.0
MEAN PER CENT RECOVERY AND DECAY	1.76	0.56	0.21	0.09	3.2	1.48	0.12	0.08	0.06	3.2

a. Based on volume of 20 milliliters.
b. Cell-infecting units; volume of inoculum used for assay was 0.2 milliliter. Mean of two determinations.
c. Mouse intracerebral LD_{50}; volume of inoculum used for assay was 0.03 milliliter. Ten mice were inoculated per dilution.
d. Insufficient number of mice died; LD_{50} could not be estimated.

For each test, a virus cloud was formed by disseminating five milliliters of infective whole-egg suspension with an FK-8 gun into a 1500-liter rotating drum (3.5 rpm). The internal environment of the drum was 25°C and 20 per cent RH. One pool of virus suspension was employed for all tests. The total concentration of virus suspension that was disseminated, estimated by the techniques of fluorescent cell-counting and intracerebral inoculation of mice, was 6.0×10^8 CIU and 1.5×10^8 LD_{50}, respectively. Clouds were sampled with Shipe impingers for one minute using an orifice with flowrate of 19.1 liters per minute. Impinger fluid consisted of McCoy cell nutrient medium with one to two drops of sterile olive oil.

IV. DISCUSSION

The feasibility of employing the fluorescent cell-counting technique for the quantitative assay of infective particles of psittacosis virus was established by the studies described here.

For diverse purposes, centrifugal force has been used to facilitate contact between cells and viral or rickettsial agents in different tissue culture systems.[7-10] For the assay of psittacosis virus by the fluorescent cell-counting technique, the use of centrifugal force, during the period of virus adsorption onto cell monolayers, was shown to increase the rapidity and efficiency of the process and resulted in assay values that were substantially higher than those obtained after stationary incubation. Augmentation of virus adsorption by the use of centrifugal force may be used to enhance the accuracy of determinations related to the characterization of virus inactivation rates and makes more meaningful those studies concerned with the early phases of virus multiplication that require synchronous infection of cells.

It was demonstrated further that the application of centrifugal force resulted in a proportional number of cell-infecting units of psittacosis virus consistent with the different volumes of inoculum employed. This finding is relevant to studies relying on the detection and assay of small quantities of infective virus. By increasing the volume of inoculum, the opportunity to detect virus in low concentration may be enhanced. It constitutes, therefore, a marked advantage over those tissue culture systems and animal hosts whose intrinsic features limit the amount of virus inoculum that may be practical to introduce.

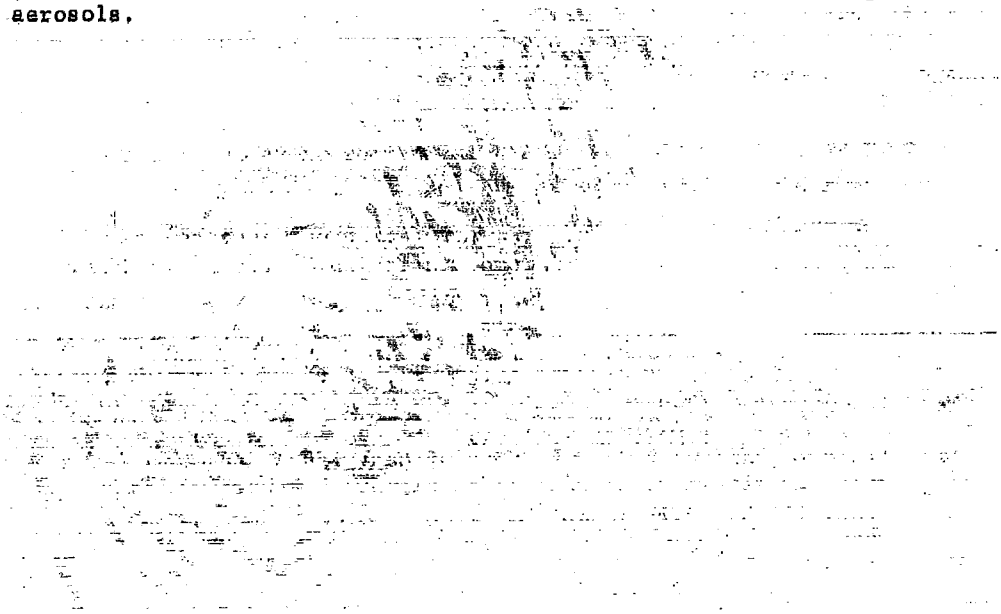
The short time required to obtain estimates of virus infectivity has been cited as an outstanding attribute of the fluorescent cell-counting procedure.[1] This was substantiated by sequential observations made during the incubation period to follow the development of psittacosis virus antigen in infected cell monolayers. Cells containing fluorescent viral antigen were easily recognized and could be enumerated within 20 to 22 hours after infection. Within this time interval of incubation, assay values obtained from infected cell cultures incubated at temperatures of 32°, 35°, 37°, and 39°C did not differ unduly, although the development of viral antigen at 32°C was retarded slightly.

In agreement with the findings of others who investigated the fluorescent cell-counting technique for assay of different viruses,[1-3] a linear function was demonstrated also between the concentration of psittacosis virus and the number of fluorescent cell-infecting units. That the distribution of infected cells on coverslip cultures did not differ significantly from the theoretical Poisson distribution paralleled the observations reported

previously with other viruses. Similarly, a high degree of precision and reproducibility was noted also in replicate assays of psittacosis virus. In sensitivity, the cell-counting procedure was comparable, if not superior, to assays that rely on dilution endpoints as an expression of virus concentration.

The demonstration of a reduction of cell-infecting units of psittacosis virus by dilutions of specific antiserum suggests the feasibility of using the fluorescent cell-counting procedure to detect and to measure serum-neutralizing antibody levels. The principal advantage for carrying out a neutralization test in this system is the relatively short time required to obtain results.

On the basis of sensitivity and rapidity of assay, the fluorescent cell-counting procedure was superior to intracerebral inoculation of mice for the assessment of aerosols of psittacosis virus. Numerous replicate assays could be performed inexpensively by the former technique by merely using additional coverslip cell cultures. By the latter method, comparable replication would involve considerable numbers of mice and an increase in animal facilities and care. The fluorescent cell-counting technique may prove useful for estimating the concentration of other viral agents in aerosols.



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